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Feature

Bacterial scissors to edit human embryos?

A recently discovered gene editing tool raises the possibility of precisely targeted changes to human genes, even in the germline. The nascent debate over the ethics and limitations of its use has already been overtaken by events. Is this a whole new Pandora's box for bioethics? **Michael Gross** investigates.

Gene therapy has recently made a surprising comeback due to the emergence of better vectors and editing tools (Curr. Biol. (2014) *24*, 983–986). The most revolutionary new tool in the kit of gene therapists is Cas9, the CRISPR-associated nuclease. Only recently discovered as part of an unexpected adaptive immune response in bacteria, this nuclease can be programmed to edit any target sequence. It opens up opportunities to edit human genes — in somatic cells and indeed in the germline — with unprecedented ease and simplicity. With these new powers come new responsibilities for scientists, and new debates over bioethics.

The bacterial immune system

Much of what we now know about the CRISPR (clustered regularly interspaced short palindromic repeats) system goes back to a collaboration between the groups of Jennifer Doudna at Berkeley, USA, and Emmanuelle Charpentier at Umea, Sweden, which started only in 2011, but has already been awarded with prestigious prizes. Both groups had independently noticed that bacterial strains seem to have a kind of memory for phages that have attacked them in the past, and together they identified the nuclease Cas9 as the crucial tool that cuts the viral genome.

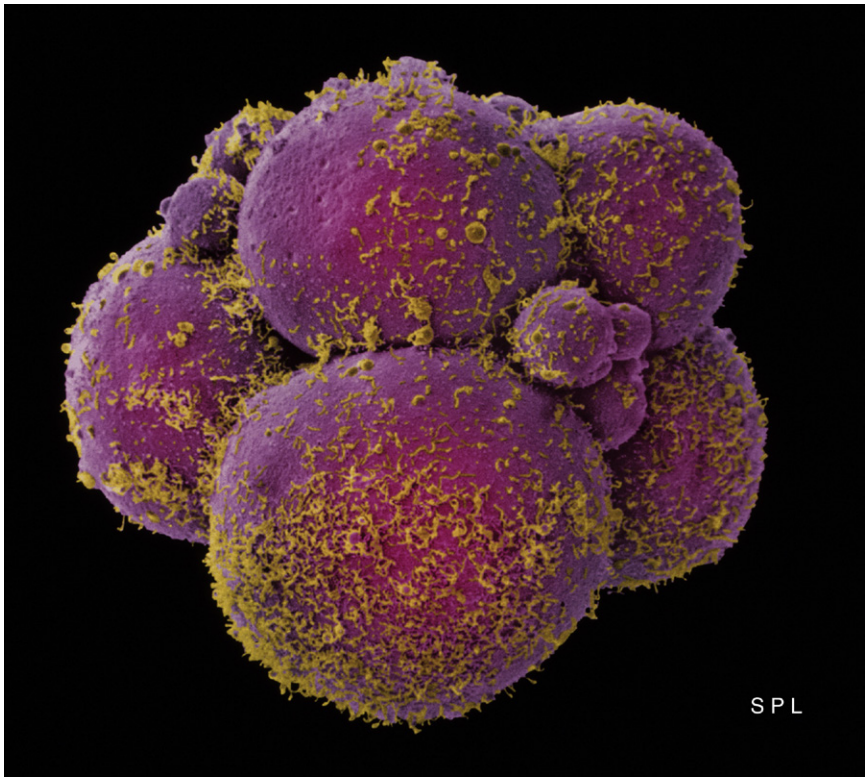
When bacteria have overcome a viral infection, they incorporate certain parts of the viral genes into the CRISPR region of their own genome as so-called spacers. These spacers are transcribed as RNAs, which then bind to the nuclease Cas9 and guide it to any intact DNA containing the very same sequence. Using additional criteria, the Cas9–RNA complex can recognise if the DNA is foreign (i.e. viral) and then cleave it to inactivate the virus. This completely unexpected system is a microbial analogue of our adaptive immune system, using RNA recognition rather than antibodies to identify known adversaries.

During the course of these sensational discoveries, the groups realised that the target recognition system, based on two separate strands of RNA, could be simplified if these strands were combined into a single, longer strand. This trick generated the new gene editing tool which turned out to be usable on just about any living cell imaginable. As geneticist Marie-Claire King wrote for *Time* magazine's list of 100 pioneers, "Emmanuelle Charpentier and Jennifer Doudna figured out the inner workings of this bacterial self-protection, and then, in a tour de force of elegant deduction and experiment, they developed a plug-and-play version of that approach."

The most recent improvement to the tool features a smaller version of the enzyme discovered in *Staphylococcus aureus*. Its gene is small enough to be delivered into cells with adeno-associated virus (AAV), currently the most widely used vector for gene therapy research (Nature (2015) *520*, 186–191).

Meanwhile, investigations on the natural function of the CRISPR system continue, as new and fascinating details of the bacterial immune system emerge, which also includes a whole range of other Cas proteins. The functional complex of these proteins with their guiding RNA molecules is also known as the Cascade complex. This comes in three different types, and two research teams have reported crystal structures of a type I complex with or without its target sequence (Science (2014) *345*, 1479–1484 and 1473–1479, respectively).

Only recently, Doudna's team could report an *in vitro* version of the *E. coli* system demonstrating that the proteins Cas1 and Cas2 are sufficient for the process of integrating the viral sequences



Dividing issue: The invention of easy-to-use genome editing tools has brought the promise of medical breakthroughs and fears of manipulations in the human germline. Lasting changes could be made in the fertilized egg or in early stage embryos like the one shown here. (Image: Dr Yorgos Nikas/Science Photo Library.)

Prizeworthy: Jennifer Doudna and Emmanuelle Charpentier identified the nuclease Cas9 as a key part of the bacterial immune response to phages and adapted it for use as a universally applicable genome editing tool. (Photo: Justin Bishop/Breakthrough Prize http://www.breakthroughprize.org.)

into the bacterial genome (Nature (2015) *519*, 193–198). In the same journal issue, the groups of David Bikard at the Institut Pasteur in Paris and Luciano Marraffini at Rockefeller University in New York, showed that Cas9 in *Streptococus pyogenes* not only acts as a nuclease, but also associates with Cas1 and Cas2 in the initial selection stage, where it ensures that the protospacers selected for the immunological memory formation are flanked by suitable protospacer adjacent motifs (PAMs), a pair of guanine bases just downstream of the target sequence (Nature (2015) *519*, 199–202).

Addressing the question of how the system manages to avoid the bacterial equivalent of autoimmune disease, i.e. attacking its own DNA, the groups of Udi Qimron at Tel Aviv University and Rotem Sorek at the Weizmann Institute, both Israel, found that the harvesting of spacers from viral DNA primarily targets DNA in replication. A number of factors, including the higher number of replication forks in viral DNA, the higher density of Chi sites in bacterial DNA, and the activity of the RecBCD double strand repair system all play a role in the avoidance of an autoimmune response (Nature (2015) *520*, 505–510).

Further work from Doudna's group has revealed the structure of a type III CRISPR complex that targets single-stranded viral RNA rather than double-stranded DNA. Similarities between this complex and the known type I complex suggest that both evolved from a common ancestor (Science (2015) *348*, 581–585). More recent research from the Marraffini lab suggests that type III complexes can target both RNA and DNA (Cell (2015) http://dx.doi.org/10.1016/j.cell.2015.04.027). High-profile publications addressing fundamental questions of the bacterial immune system are still appearing regularly, showing that our understanding of this recently discovered phenomenon is still in flux. In the meantime, its revolutionary application in biomedical science is gathering pace.

Somatic gene repair

By combining this RNA-guided gene-editing tool with the desired repair templates, the well-directed cut can enable deletions, replacements or insertions of genetic material. In principle, the technique could alter germline cells and thus the genetic make-up of future generations, as well as somatic cells, where it will only affect the present patient treated.

Somatic gene repair is less questionable on ethical grounds but still has its safety issues. When genetic material has to be introduced into many cells at once, there is always the risk that some of it may end up in the wrong place and cause side effects. (In this respect, the somatic editing can be more challenging than germline editing, which could be included in the fertilisation step.)

The first targets for somatic editing are therefore likely to be blood cells, which can easily be taken, treated and returned. One approach currently undergoing clinical trials is to inactivate the receptor to which HIV docks — an idea inspired by the rare patients who are naturally resistant to HIV. The hope is to free patients of the need to take antiretroviral drugs. The company Sangamo BioSciences at Richmond, California, currently tests such a therapy with the older zinc finger nuclease technology, but it could also point the way for similar applications of CRISPR nucleases, which could be much more rapidly adapted to new targets.

Other conditions that could be treated in similar ways include β thalassaemia, sickle cell anaemia, haemophilia, Huntington's disease and SCID (severe combined immunodeficiency) — the condition that was the target of early attempts at gene therapy in the 1990s.

The CRISPR–Cas system also promises better control in the study of disease states in stem-cell-derived *in vitro* systems. So far, such studies have been hindered by confounding genetic variability. However, CRISPR now enables researchers to very precisely repair the particular mutations thought to be involved in order to create so-called isogenic cell lines that are genetically identical to their diseased counterparts at every locus except the putative causative gene, as Paul Fairchild, co-director of the Oxford Stem Cell Institute at the University of Oxford, UK, points out. Experiments linking specific gene repairs to amelioration of disease states could bring unequivocal proof of causation. "There is little doubt that combining powerful technologies such as CRISPR and induced pluripotency will greatly accelerate our understanding of the genetic basis of disease processes that have remained intractable for decades," Fairchild comments.

Germline operations

The envisioned manipulations of somatic cells and *in vitro* cell cultures may not raise many concerns at the current state of bioethical debates. What did cause a big stir, however, was the more troubling possibility that the new editing tool might be applied to the germline and thus

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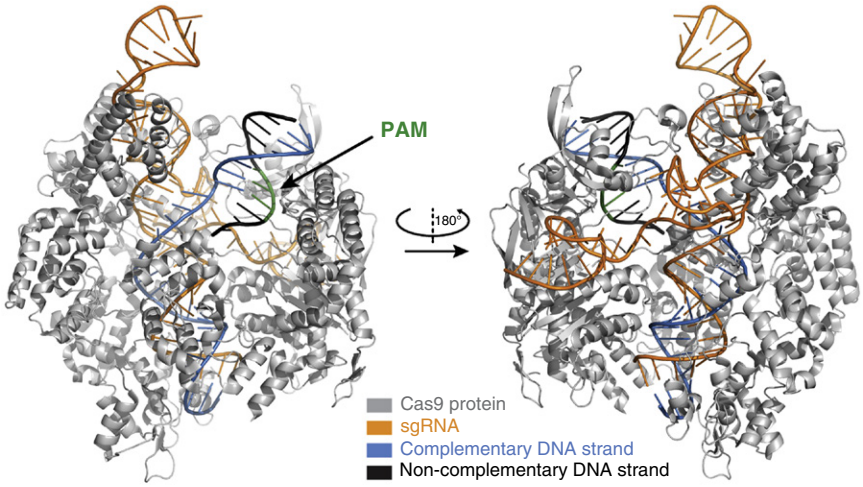
alter the genes of future generations. Germline manipulation has been a fundamental taboo so far — but one that was easily policed as long as there was no promising technology with which to manipulate the germline.

Now the situation is entirely different. The technology that might one day lead to designer babies as envisioned in the film GATTACA is on the table, and it will be impossible to uninvent it. A small door towards germline manipulation was already opened in February 2015, when the UK, as the first country in the world, legislated to allow the use of mitochondrial replacement. The law will come into force in October, by which time the regulatory body, the Human Fertilisation and Embryo Authority (HFEA), will draw up detailed procedures regarding how it will regulate the application of the new technique.

Rumours of several research papers applying CRISPR editing to human embryos were circulating early this year, prompting the International Society for Stem Cell Research (ISSCR) and two informal groups of experts to voice their concern and call for moratoria and a wider debate on the fundamental ethical aspects.

On March 19th, the ISCCR called for "a moratorium on attempts to apply nuclear genome editing of the human germline in clinical practice. Scientists currently lack an adequate understanding of the safety and potential long term risks of germline genome modification." Further, the organisation asserted "that a deeper and more rigorous deliberation on the ethical, legal and societal implications of any attempts at modifying the human germline is essential if its clinical practice is ever to be sanctioned."

A week later, Edward Lanphier, the president and chief executive of the above-mentioned company Sangamo BioSciences, and others published a comment in Nature asking researchers to abstain from editing the human germline (Nature (2015) *519*, 410–411). The authors argued that all genuine medical needs are better served by other approaches, such as prenatal genetic diagnostics and IVF. After acknowledging the spectacular impact of CRISPR on gene editing, they wrote: "But we cannot imagine a situation in which its use in human embryos would offer a therapeutic benefit over existing and developing methods."



Bacterial scissors: Cas9, the enzyme at the heart of the CRISPR gene editing system, uses a strand of RNA to identify the target site. As this RNA can be readily exchanged, Cas9 can be targeted to any location in a genome. (Image: Anders *et al*. (2014). Structural basis of PAM-dependent target DNA recognition by the Cas9 endonuclease. Nature *513*, 569–573, http://dx.doi.org/10.1038/nature13579.)

The authors expressed their concern that a bioethics backlash against the use of germline editing might harm the prospects of other forms of gene therapy that are currently under development and appear much more promising. "Legitimate concerns regarding the safety and ethical impacts of germline editing must not impede the significant progress being made in the clinical development of approaches to potentially cure serious debilitating diseases," Lanphier and colleagues concluded.

A separate statement was published a week later by a group of experts who met at Napa, California, in January, including Jennifer Doudna, George Church, and David Baltimore (Science (2015) *348*, 36–38). This comment, under the title "A prudent path forward for genomic engineering and germline gene modification" calls for a moratorium on germline modification while safety and ethical concerns are being discussed, but does not rule out its use at a later stage.

The authors called for further research to address questions like the likelihood of off-target modifications and the physiological effects in cells after gene editing. They recommended to "strongly discourage, even in the countries with lax jurisdictions where it might be permitted, any attempts at germline genome modification for clinical application in humans, while societal, environmental, and ethical implications of such activity are discussed among scientific and governmental organizations." However, this group left the door open for scientists to embark on germline modification in the future, if safety, transparency, and public trust can be maintained.

Only two weeks later, the rumours were confirmed when the groups of Canquan Zhou and Junjiu Huang at Sun Yat-sen University in Guangzhou, China, published the first report of germline editing in human embryos (Protein Cell (2015) *6*, 363–372). In an attempt to score their first without being vilified for transgression of ethical boundaries, the researchers used tripronuclear zygotes for their experiments. These result when an egg is fertilised by two sperms simultaneously. They are not viable and would normally be discarded in IVF clinics. Thus, their manipulations only affect a dead end of the germline and could under no circumstances end up affecting live human beings.

That said, the experiments aimed at the gene involved in β thalassaemia, so they were clearly conducted with the ultimate ambition of medical applications. The results of the study show, however, that the path to a successful germline modification, should humanity decide to pursue it, may still be a long trek.

Specifically, the desired genetic manipulation succeeded only in four out of 28 embryos, and even those embryos showed mosaicism, meaning that not all

of their cells carried the change. Moreover, the cells had their own mind on how to repair the change, with a quarter of them using endogenous templates, while cuts in off-target locations were also observed. The high likelihood of unwanted reactions led the authors to conclude that the system is not ready for clinical application yet. Ironically, they backed up this conclusion by citing both the statements of concern from Lanphier and colleagues and from the Napa meeting, which had been triggered by the rumours of their own endeavours.

Criticism of the publication has claimed that the method used was not up-to-date and that the paper was rushed through publication, with an acceptance date only one day after receipt. However, the journal has defended the review process and stated that the paper arrived with peer reviews from previous submissions to other journals and that it was fast-tracked due to its high relevance.

While the Chinese paper shows that designer babies are not going to be born soon, it has also alerted the world to the realisation that, once the technical issues are resolved, it may be impossible to police a global ban on germline modifications. Even if most of us don't want to live in a world of genetically optimised offspring as described in GATTACA, the impact of technological progress may already be driving us in that direction.

The Nuffield Council on Bioethics, which has already played a key role in shaping the UK's policy to permit mitochondrial replacement therapy with a report published in 2012, is now setting up a new project to explore the ethical issues attached to genome editing. The council's assistant director, Peter Mills wrote in a blog post: "The escape of genome editing from the laboratory into the public sphere — especially following the development of the CRISPR–Cas9 system — suggests that contained use, at least in the sense of reserving questions about the use of genome editing for researchers *qua* researchers to address, is no longer possible." Pandora's box has been cast wide open, and we, as a civilisation, now face the challenge of deciding how we are going to deal with its content.

Michael Gross is a science writer based at Oxford. He can be contacted via his web page at www.michaelgross.co.uk

Q & A

Barbara Shinn-Cunningham

Barbara Shinn-Cunningham is a Professor of Biomedical Engineering at Boston University. She graduated from Brown University in 1986 and earned her Ph.D. from the Massachusetts Institute of Technology in 1995. She joined the faculty of Boston University in 1997, initially as a member of the now-defunct Department of Cognitive and Neural Systems. She is a Fellow of the Acoustical Society of America, a Fellow of the American Institute for Medical and Biological Engineers, and a lifetime National Associate of the National Research Council. She is currently Director of the Center for Computational Neuroscience and Neural Technology, which promotes interdisciplinary research at Boston University. Her research uses behavioural, neuroimaging and computational methods to study auditory attention, individual differences in hearing ability, cross-modal interactions, and spatial hearing, both in healthy adult populations and in individuals facing various challenges, including hearing loss, autism, and blast injury.

What turned you on to biology in the first place? Actually, the only biology class I ever took was when I was 15, in high school. I studied electrical engineering and mathematics as an undergraduate, then went to graduate school at MIT thinking I would learn to design computers. Once there, I discovered groups of engineers studying auditory perception, speech, and related areas. As a semi-serious musician (I play oboe and English horn), I was immediately seduced by the idea of studying hearing. Well, actually, not immediately. I spent a couple of weeks soul searching. I had always thought of myself as a hard-core engineer, doing heavy-duty mathematics. To switch to an area that was just plain fun felt wrong, as if I was selling out somehow. But I got over that. And I have been having fun ever since. With each step of my career, I've been drawn to looking deeper and deeper into how information is encoded in the brain. Somehow, I now find myself a neuroscientist. But I still suffer from gaps in my knowledge. That is why I like collaborating.

How did you end up studying auditory attention? My career path is a series of happy coincidences. As a master's student at MIT, I did my first research project, on spatial hearing, under the guidance of Nat Durlach, Steve Colburn, and Pat Zurek (three fantastic mentors and an all-star team in the world of hearing). I worked briefly at MIT Lincoln Laboratory as a hardware engineer, but decided I really liked pure research, so I went back to MIT to do my PhD in the same research group. My son Nick was born three weeks after I defended my thesis. I spent the next two years working as a part-time post-doc, joining the faculty at Boston University when my son Will was two months old.

Because there was no anechoic chamber at Boston University, I couldn't do the carefully controlled studies of sound localization I had been doing at MIT. So instead, like making the proverbial lemonade from lemons, I began studying how room acoustics affect auditory spatial cues and how we localize sounds in the real world. I was struck by how messy and noisy spatial auditory cues typically are (nothing like the textbooks suggest). This observation got me interested in the fact that spatial hearing has a big impact on our ability to communicate in everyday settings, even though the localization cues are unreliable,

